Supplementary Material (ESI) for Dalton Transactions

Supporting Information

One and two photon fluorescent complexes of rhenium and their technetium analogues

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1) Chemicals and Instrumentation

Unless otherwise stated, all reagents and solvents were ACS grade or higher and used without further purification. All manipulations and reactions were carried out in air unless otherwise noted. Dry solvents were obtained from an MB-SPS-800 MBROWN solvent purification system. Ligands L^1, L^3 [1], $[ReCl_2(NNAr)(MeCN)(PPh_3)_2]$ (Ar = Ph, C_6H_4OMe-4),[2] $[ReBr(CO)_5]$[3] and $[ReO_4]^-$ [4] were prepared as previously described and $[TcO_4]^-$ [5] was used as obtained from the generator.

Elemental analyses were performed by the microanalysis service of the department at the University of Oxford. NMR spectra were recorded on either a Varian Mercury VX300 spectrometer, (^{1}H 300 MHz, $^{13}C\{^1H\}$ at 75.5 MHz) or a Varian Unity 500 MHz spectrometer, (^{1}H 499.9 MHz, $^{13}C\{^1H\}$ at 125 .7 MHz) referenced to residual protic solvent. Mass spectra were recorded on a Micromass LCT Time of Flight Mass Spectrometer using positive ion electrospray (ES^+), solid probe electron impact (EI) or field ionization (FI^+) techniques. Where possible accurate masses are reported to four decimal places using tetraoctylammonium bromide (466.5352 Da) as an internal reference. The UV/vis spectra were recorded on a Cintra 10 UV/vis spectrometer.
High performance liquid chromatography (HPLC) was conducted using a Gilson HPLC machine with a Hamilton PRP-1 reverse phase column and UV/vis detection at 254 nm. Retention times, using a water/acetonitrile gradient elution method are presented for the compounds. All absorbance spectra were recorded using a Perkin Elmer λ19 UV-visible spectrometer. Measurements were performed in double beam mode baseline corrected in matched quartz cuvettes. Stock solutions of **1-3, 5 ,9 and 10 in DMSO** were prepared to give 0.2 M solutions. The absorbance spectrum of **10** was recorded in chloroform.

2) Synthetic Procedures

*Synthesis of **1-3***

To an ethanolic solution (20 mL) of $[NH_4][ReO_4]$ (0.100 g, 0.4 mmol), conc. HCl (37%, 1.0 mL) was added drop-wise. To this solution $SnCl_2$ (0.212 g, 1.1 mmol) in ethanol (5 mL) was added drop-wise followed by the addition of the appropriate ligand $\mathbf{L^{1-3}}$ (0.4 mmol) and the color of the reaction turned instantly to turquoise blue. The addition of diethyl ether (30 mL) gave a blue precipitate which was isolated by filtration (80%). *(Characterisation data for these complexes were identical to that described before).*[6]

*Synthesis of **4***

$[NH_4][ReO_4]$ (0.100 g, 0.4 mmol) and acetylhydrazine (0.0275 g, 0.4 mmol) were stirred in CH_3CN (10 mL) and HCl (0.2 mL, 12M) was added drop-wise. During this time a yellow solution was formed. *Meso*-dimercaptosuccinic acid (0.068 g, 0.4 mmol) was added to the mixture and the reaction was heated under reflux for 30 min. $\mathbf{HL^1}$ (0.071 g, 0.4 mmol) was added and the reaction was heated under reflux for another 30 min to give a brown air stable solid which was isolated by filtration and washed with diethyl ether (3

x 30 mL) and dried *in vacuo* (70%). (Found: C, 25.3; H, 2.2; N, 10.0. $C_{12}H_{11}N_4O_7ReS_2$ requires C, 25.1; H, 1.9; N, 9.8 %); IR ν/cm^{-1} (KBr) 3128 (br, NH_4^+, OH), 1704 (s), 1672 (s, C=O), 1404 (s), 1248 (m), 1208 (m), 1704 (m), 966 (m, Re=O), 656 (w); δ_H (300 MHz; ppm; d_6-DMSO; Me_4Si) 11.39 (2), 10.69 (1), 9.08 (1) (br, s, OH), 7.93 (2), 8.67 (2) (d, 2H, 3-pz), 7.54 (2), 8.62 (1) (d, 2H, 5-pz), 7.94 (2), 7.37 (1) (dd, 2H, 4-pz), 7.02 (s, 1H, CH), 4.72 (2), 4.53 (1) (br, s, 2H); $\delta_{13C\{1H\}}$ (75.5 MHz; ppm; d_6-DMSO; Me_4Si) 174.2 (C=O, dmsa), 172.3 (C=O, **HL[1]**), 140.4 (C-3pz), 131.2 (C-5pz), 107 (C-4pz), 74 (CH, **HL[1]**), 23.2 (2), 21.8 (1) (CH, dmsa); *m/z* (ESMS)(+) 574.9911 $[M]^+$. $C_{12}H_{11}N_4O_7ReS_2$ requires 574.9702, HPLC **4** (dissolved in H_2O) (gradient elution CH_3CN - H_2O, 5 - 95) 1.8 min; HPLC (flow rate 0.3 mL min^{-1}), (gradient elution, A – B) (A = 0.1% trifluoroacetic acid in H_2O, B = 0.085% trifluoroacetic acid in CH_3CN : H_2O, 70 : 30) 1.8, 1.6, 6.8 min.

*Synthesis of **5***

A solution of $[ReBr(CO)_5]$ (0.1 mmol) and **HL[3]** (0.1 mmol) and ^{t}BuOK (0.1 mmol) in CH_3CN (50 mL) were heated under reflux for 3 hours. After this time a white precipitate was obtained which was isolated by filtration. The product was washed with Et_2O (3 x 30 mL) and dried *in vacuo* to give complex **6** as a white solid (78%). UV-vis (MeCN; 1 mM) 250 (ε = 2750), 287.5 nm (ε = 2350); IR ν_{max}/cm^{-1} (KBr) 2944 (s, br), 2864 (br, s), 2592 (w), 2046 (s), 1954 (s), 1920 (s), 1464 (s), 1408 (m), 1382 (m), 1262 (m), 1382 (m), 1262 (m), 1102 (m), 1049 (m), 860 (m), 775 (m), 614 (w); *m/z* (ESMS)(+) 928.1318 (100%), ($[M + K]^+$. $C_{39}H_{34}KN_4O_9Re$ requires 928.1520); HPLC, **6** (dissolved in MeCN), (isocratic elution CH_3CN – H_2O, 80 : 20) 3.5 min; (gradient elution CH_3CN – H_2O, 5 – 95) 19.3 min.

*Synthesis of **9***

In a round bottom flask (250 mL), potassium tertiary butoxide (0.036 g, 0.3 mmol), **HL[3]** (0.3 mmol) and $[ReCl_2(N_2Ph)(NCMe)(PPh_3)_2]$ (0.300 g, 0.3 mmol) were heated under reflux in MeOH (60 mL) for 4 h. The orange precipitate formed was filtered off and washed with MeOH (3 x 40 mL) and dried *in vacuo* (72%). (Found: C, 59.9; H, 4.4; N, 6.9. $C_{60}H_{51}ClN_6O_6PRe$ requires C, 59.8; H, 4.3; N, 7.0%); UV-vis (DMSO; 1 mM) 230, 290; IR ν_{max}/cm^{-1} (KBr) 3446 (br), 3223 (w), 3121 (w), 3018 (w, br), 2896 (w), 2452 (w), 2161 (w), 1881 (w), 1698 (s, C=O), 1580 (s, NN); 1467 (s), 1458 (m), 1404 (m), 1387 (m), 1321 (w), 1282 (m), 1241 (m), 1174 (m), 1104 (m), 1008 (w), 994 (w), 891 (w), 862 (s), 784 (s), 763 (s), 741 (s), 622 (w), 590 (s); δ_H (300 MHz; ppm; d_6-DMSO; Me_4Si) 8.22 – 7.75 (m, 15H, PPh_3), 7.74 – 7.70 (m, 5H, N_2-Ph), 7.68 – 7.31 (m, 16H, Ph), 6.35 (dd, 2H, 4-pz), 3.22 (s, 6H, CH_3), 3.28 (s, 6H, CH_3); $\delta_{13C\{1H\}}$ (75.5 MHz; ppm; d_6-DMSO; Me_4Si) 164.10 (C=O), 145.6 – 135.71 (PPh_3), 141.21 (C-3pz), 133.25 – 130.10 (N_2Ph), 120.81 (C-5pz), 105.22 (C-4pz), 42.23 (CH_3), 40.38 (CH_3); $\delta_{31P\{1H\}}$ (ppm; d_6-DMSO; H_3PO_4) 10.92; *m/z* (ESMS)(+) 1204.3400 (100%), ($[M]^+$. $C_{60}H_{51}ClN_6O_6PRe$ requires

1204.2849); HPLC **3.2** (dissolved in DMSO), (gradient elution $CH_3CN–H_2O$, 5 - 95) 15.7 min.

*Synthesis of **10***

Prepared in an analogous procedure to that used for **6** (74%). (Found: C, 59.4; H, 4.3; N, 6.9. $C_{61}H_{53}ClN_6O_7PRe$ requires C, 59.4; H, 4.3; N, 6.8%); UV-vis (DMSO; 1 mM) 235 (ε = 2300), 288 (ε = 1750), 383 nm (ε = 650); IR ν_{max}/cm^{-1} (KBr) 3412 (w, br), 3261 (w), 3152 (w), 2635 (w), 2582 (w), 2420 (w), 2106 (w), 1987 (w), 1885 (w), 1773 (w), 1664 (s, C=O), 1584 (s, NN), 1445 (m), 1412 (m), 1382 (w), 1356 (w), 1281 (w), 1081 (m), 843 (w), 821 (m), 786 (s), 769 (s), 742 (s), 665 (w); δ_H (300 MHz; ppm; d_6-DMSO; Me_4Si) 8.18 – 7.66 (m, 15H, PPh_3), 7.72 – 7.64 (m, 4H, N_2-Ph), 7.56 – 7.28 (m, 16H, Ph), 6.41 (dd, 2H, 4-pz), 3.33 (s, 3H, $NNC_6H_4OCH_3$), 3.21 (s, 6H, CH_3), 3.17 (s, 6H, CH_3); $\delta_{13C\{1H\}}$ (75.5 MHz; ppm; d_6-DMSO; Me_4Si) 168.2 (C=O), 140.2 – 136.3 (PPh_3), 138.2 (C-3pz), 129.3 – 127-5 (N_2Ph), 117.5 (C-5pz), 106.3 (C-4pz), 44.2 ($NNPhOCH_3$), 43.7 (CH_3), 41.8 (CH_3); $\delta_{31P\{1H\}}$ (ppm; d_6-DMSO; H_3PO_4) 10.80; *m/z* (ESMS)(+) 1240.3021 (100%), ($[M + Li - H]^+$. $C_{61}H_{52}ClN_6O_7PReLi$ requires 1240.3021); *m/z* (ESMS)(+) 1240.3021 (100%), ([M + Li – H]. $C_{61}H_{52}ClN_6O_7PReLi$ requires 1240.3021); HPLC **7** (dissolved in DMSO), (gradient elution $CH_3CN–H_2O$, 5 - 95) 11.9 min.

Caution! ^{99m}Tc is a γ-emitter (Eγ = 0.23 MeV, $t_{1/2}$ = 6 h)

All manipulations were performed in laboratories approved for low-level radioactivity using monitored hoods and glove-boxes. Normal radiation safety procedures were used at all times, to prevent contamination and inhalation.

Synthesis of complexes 6,7

A glucoheptonate kit (lypophilized) (Techne/Demoscan, NCSR "Demokritos") containing sodium glucoheptonate (50 mg) and stannous chloride (0.1 mg) was mixed with [$^{99m}TcO_4$] (3 mL, 30 mCi) to give ^{99m}TcO-glucoheptonate. The solution of ^{99m}TcO-glucoheptonate (0.5 mL) was added to an ethanolic solution (0.5 mL) of L (1 mg). To this mixture HCl (120 μL, 1N, pH = 3) was added. Samples of the above solutions were analyzed by radio HPLC.

Following this method we have prepared [$^{99m}TcOCl_2L^1$] **7** and [$^{99m}TcOCl_2L^3$] **7**. The radiochemical yields of the new ^{99m}Tc(V) complexes **6** and **7** were monitored by HPLC (gradient elution $CH_3OH – H_2O$, 5 – 95). The chromatographic profile of complex **7** displays a major radiochemical species as a single peak with a retention time of 19.60 min (82%) with a less intense peak with a retention time of 2.9 min (18%) assigned to [$^{99m}TcO_4$].
The HPLC of the corresponding rhenium surrogate compound [$ReOCl_2L^3$], **3** allowed for macroscopic identification of the radiochemical conjugate with a retention time of 19.70 min (84.09%) and a less intense peak with a retention time of 11.37 min (15.86%). Complexes **6-7** were obtained using ligand concentrations 10^{-5} M. In both cases, the radiolabelling yield was between 0 – 5 % at alkaline pH, between 30 – 40 % at neutral pH and greater than 80 % at acidic pH (3.0).

Synthesis of 8

[$^{99m}TcO_4$] (1 mL) was added to an Isolink kit to prepare [$^{99m}Tc(CO)_3(H_2O)_3]^+$. An ethanolic solution (0.1 mL) of **HL^3** (0.06 mg) was added to [$^{99m}Tc(CO)_3(H_2O)_3]^+$ (0.4 mL). The mixture was incubated for 20 min at 60°C and was analyzed by HPLC.
The complex **8** was obtained by reaction of the *fac*-[$^{99m}Tc(H_2O)_3(CO)_3]^+$ synthon with **HL^3** in greater than 90% radiochemical purity as identified by HPLC. Chromatograms showed a major radiochemical peak with a retention time of 19.42 min in 84.73% radiochemical yield (**Figure 1**). The peak with a retention time of 2.06 min (15.27%) corresponded to [$^{99m}TcO_4$], also present in the [$^{99m}Tc(CO)_3(H_2O)_3$] starting material, with the actual yield for complex **8** being greater than 95%.

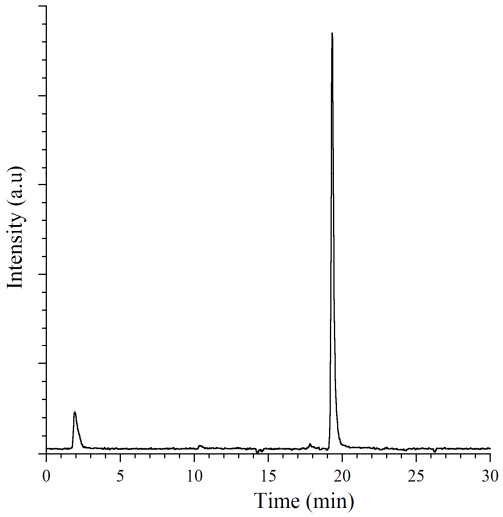

Figure 1: HPLC elution profile of the new radiolabelled complex [$^{99m}Tc(CO)_3L^3$], **8** with a retention time of 19.42 min.

The new ^{99m}Tc radiolabelled complex **8** was identified by comparison of the HPLC chromatogram with that of its Re surrogate, [$Re(CO)_3L^3$], **5**. Under the same conditions the HPLC of **5** showed formation of a single species with a retention time of 19.83 min.

The radiochemical yield for all ^{99m}Tc complexes was >95 % with final concentration of the ligands spanning from 10^{-4} to 10^{-5} M.

3) Fluorescence Studies

Absorbance Spectra and Extinction Coefficients

4) Emission Spectra and Quantum Yields

Fluorescent measurements of complexes **1-3**, **5** and **9-10** were performed with a Hitachi, F-4500 Fluorescence Spectrometer. Emission was monitored from 220 to 900 nm. Samples of were prepared in acetonitrile. The emission spectra of **1** and **9,10** are shown

in **Figures 2** and **3** respectively. Naphthalene standard was used to measure the quantum yields and was prepared in a concentration of 0.1 M and diluted. **Table 1** summarizes the absorption and emission properties of $\mathbf{HL^3}$ and complex **3**. **Table 2** summarizes absorption and emission properties for complexes **6-8**.

Table 1: Absorption and emission wavelengths of $\mathbf{HL^3}$ and complex **3** dissolved in CH_2Cl_2 and CH_3CN at room temperature with excitation at 260 nm and emission at 339 nm and their quantum yields.

Compound	$\Phi(CH_3CN)$	Φ(DCM)	Absorption (ε)
$\mathbf{HL^3}$	0.98	0.89	210 (97,000), 248 (48,000), 295 (32,000)
3	0.94	0.85	293 (92,000), 350 (35,000), 670 (200)

$\Phi_s = \Phi_r (D_s/D_r) (A_r/A_s) (n_s/n_r)^2 (I_r/I_s)$; r = reference, s = sample, Φ = quantum yield, D = integrated area under emission spectrum, A = absorbance of solution at excitation wavelength, n = refractive index of pure solvent, I = intensity. A graph of integrated fluorescence intensity *vs* absorbance was a straight line.

Emission was measured between 200 and 500 nm with an excitation at 366 nm.
In summary, the introduction of phenyl or $C_6H_4OCH_3$ groups at the 3- and 5-positions of the pyrazole rings in bis(pyrazol-1-yl)acetic acid caused intense fluorescence of the corresponding ligands $\mathbf{HL^2}$ and $\mathbf{HL^3}$ in solution. The rhenium complexes of these ligands **1-3** are also strongly fluorescent in solution exhibiting the same emission properties.

5) Two-Photon Fluorescence Measurements

The two-photon set up used in this study has been previously described (Botchway et al., 2008)[7]. Briefly, the two-photon ultraviolet transmitting microscope was constructed in the Central Laser Facility of the Rutherford Appleton laboratory using custom made XY galvanometers (GSI Lumonics) for the scanning system. Laser light at a wavelength of 560-630 nm, 200fs, 75 MHz pulses was obtained from an optical parametric oscillator (OPO, APE-Coherent) pumped by a mode-locked titanium sapphire laser (Mira, Coherent Lasers) producing 180 fs pulses at 75 MHz. The laser beam was focused to a diffraction-limited spot through a water-immersion ultraviolet corrected objective (Nikon VC, NA 1.2) and specimens illuminated at the microscope stage of a modified Nikon TE2000-U with modified UV transmitting optics. Fluorescence emission was collected in the descanning mode, bypassing the scanning system, and passed through a 340 nm interference filter (U340, Comar). The scan was operated in the normal mode and line, frame and pixel clock signals were generated and synchronized with an external fast microchannel plate photomultiplier tube (Hamamatsu R3809U) used as the detector. These were linked via a Time-Correlated Single Photon Counting (TCSPC) PC module SPC830 (Becker and Hickl, Germany). For studies in solution, samples were excited at a stationary point without scanning the laser and the fluorescence decay was collected from the same point. Image Analysis For the two-photon cell imaging studies, Grey scale images (6 bit, 256 3 256 pixels) were exported from Becker and Hickl software as bitmaps and converted into TIFF files at the same pixel threshold and presented here without further image processing. Fluorescence lifetime imaging microscopy of cells loaded with metal complex were analyzed using the SPCImage analysis software (Becker

and Hickl, Germany). A thresholding function within the FLIM analysis software ensured that non correlating photons arriving at the detector were not included in the analysis.

6) *In Vitro* Stability Studies

The stabilities of complexes **1-3** were monitored in phosphate buffer solutions (PBS) (pH = 7.4) at 37°C over 24 h. The samples were analyzed by HPLC at 1, 2, 4, 6 and 24 h, and the results obtained have confirmed a high stability for the complexes. The stabilities of complexes **1-3** were also determined by HPLC in a DMSO-PBS (0.2 M) (5 : 95) solution containing a molar excess of cysteine hydrochloride (10 mM) at pH 7.4. Samples were incubated at 37°C and aliquots were analyzed by HPLC at successive intervals in a 24 hour period. A representative HPLC chromatogram for **3** shows the presence of the complex with a retention time of 5.5 min which confirms the stability of the complex over 24 hours. Formation of other species, indicating decomposition of the compound, were not detected and the observed decrease in the intensity of the peak with a retention time of 5.5 min being due to slow precipitation of the complex **3** in the PBS buffer due to limited solubility. The peak with retention time of 1.75 min corresponds to the solvent, DMSO.
In vitro stability studies of **7** were carried out and HPLC traces of the complex were collected after 30 min, 1 h and 4 h indicating that 82%, 75% and 42% of the complex remained intact. The increase in intensity of peak at 3 min suggests hydrolysis of the complex to $[TcO_4]^-$. This observation is not surprising considering the labile nature of the chloride ligands and indicates that the stability of the complex is insufficient for radiopharmaceutical use.

7) X-ray Crystallography

The full details of the structure determination appear in the *cif* file associated with this paper.

*X-ray Crystal Structure of **5***

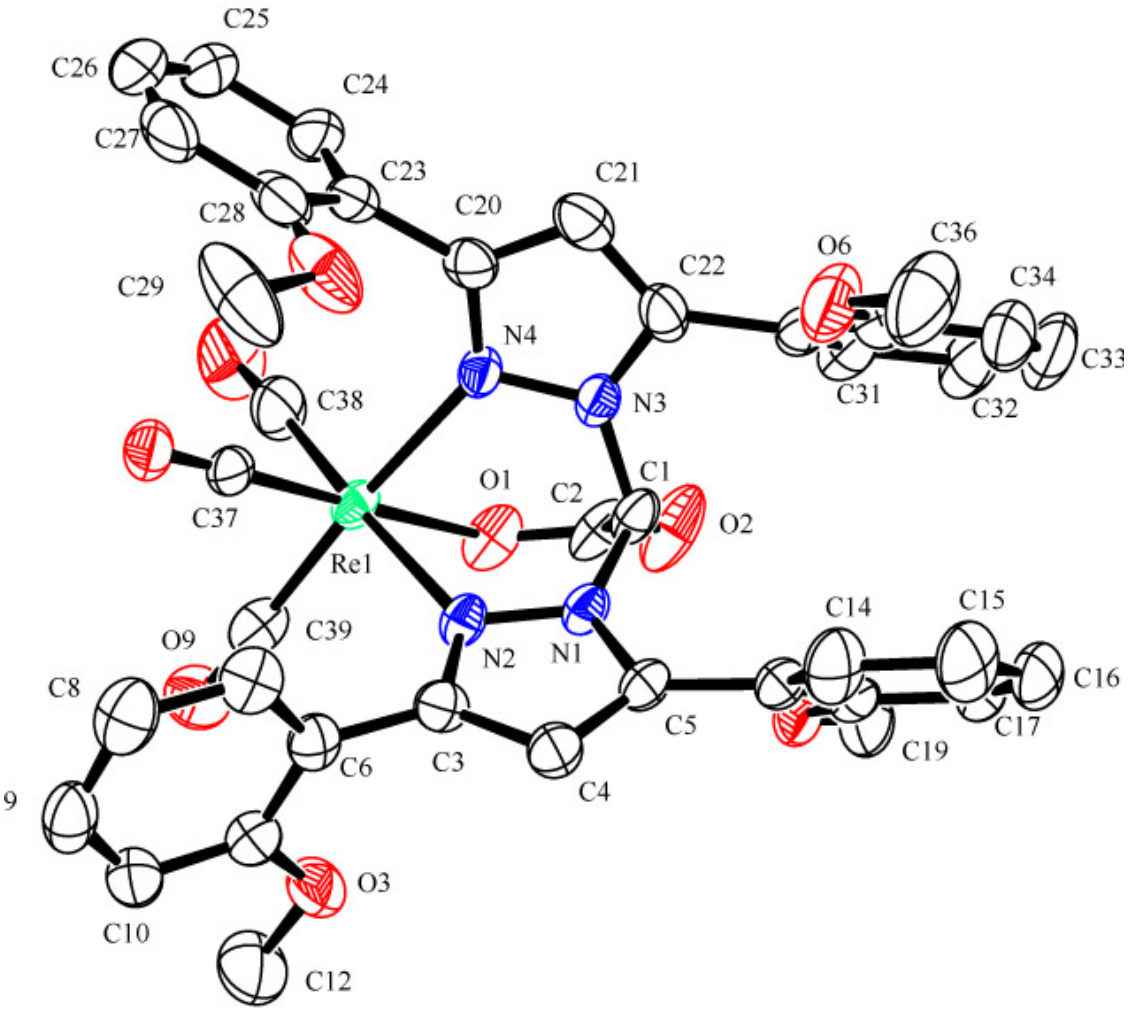

Figure 2: Representation of the molecular structure of complex **5**.

References Supplementary Information

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